FINDING OF NO SIGNIFICANT IMPACT

ENVIRONMENTAL ASSESSMENT OF USAMRMC-FUNDED RESEARCH AT VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Expression of *Brucella* Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice

1. PROPOSED ACTION: The proposed action (preferred alternative) and subject of this Environmental Assessment (EA) is the conduct of a proposed research project for the U.S. Army Medical Research and Materiel Command (USAMRMC) at the Virginia Polytechnic Institute and State University (VPI&SU). The proposed study is part of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats. *Brucella* is a potential biological warfare threat for which there currently is no acceptable human vaccine. The research project – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* – will be conducted in existing facilities at the VPI&SU in Blacksburg, Virginia. Researchers will prepare and investigate strains of *Brucella melitensis*, one of the causative agents of human brucellosis, for their potential use in vaccines. The EA is incorporated by reference in this Finding of No Significant Impact (FNSI).

2. ALTERNATIVES CONSIDERED: During the preparation of this EA, one alternative to the proposed action was identified. This alternative is to cease funding of the research proposed by VPI&SU (Alternative II, no action).

3. ENVIRONMENTAL CONSEQUENCES AND MITIGATION MEASURES: It is unlikely that significant adverse environmental impacts will result from implementing the proposed action. The preferred alternative includes adherence to existing regulations and standards and the use of specialized facilities. Adherence to health, safety, and environmental regulations applicable to the conduct of research involving biohazardous microorganisms will mitigate risk to the workforce and ensure environmental protection.

4. FACTORS CONSIDERED IN THE FINDING OF NO SIGNIFICANT IMPACT: The EA systematically reviews the nature of the proposed action and associated risks and issues. Particular attention is given to protection of the workforce and surrounding community. Alternatives with regard to needs of the U.S. and the U.S. Army and potential adverse effects on the environment are evaluated.

5. CONCLUSIONS: The principal conclusions of this EA are: (1) the conduct of the proposed research project – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* – (Alternative I, preferred alternative) is not expected to result in significant adverse environmental impacts; (2) implementation of the preferred alternative will likely result in important benefits to the U.S. by enhancing progress toward developing an effective vaccine against human brucellosis; and (3) ceasing the proposed research project (Alternative II, no action) will eliminate the negligible environmental impacts associated with conducting the research, but it will also eliminate the potential for making significant advances in developing a vaccine against human brucellosis.

FOR THE COMMANDER

CLAUDIA BARTZ
Colonel, Army Nurse Corps
Deputy Chief of Staff for Regulatory Compliance and Quality
U.S. Army Medical Research and Materiel Command

Comments on this FNSI may be directed to Commander, USAMRMC, ATTN: MCMR-PA Charles Dasey, Fort Detrick, MD 21702-5012 and must be received by August 31, 1998. Copies of the EA are available for review by the public at the Montgomery-Floyd Regional Library, Blacksburg Branch, 200 Miller Street, Blacksburg, VA 24060, and the Newman Library, VPI&SU, Blacksburg, VA, 24062 and at http://MRMC-www.army.mil. Copies may also be obtained by writing to Commander, USAMRMC, ATTN: MCMR-RCQ-E Robert Carton, Fort Detrick, MD 21702-5012.

ENVIRONMENTAL ASSESSMENT
of
United States Army Medical Research and Materiel Command (USAMRMC)-Funded Research at Virginia Polytechnic Institute and State University

Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice

Prepared by:
United States Army Medical Research and Materiel Command
Fort Detrick, MD 21702

With Technical Assistance from:
BSA Environmental Services, Inc.
21403 Chagrin Boulevard, Suite 101
Beachwood, OH 44122

Under subcontract to:
Science Applications International Corporation
Frederick, MD 21703
Contract Number DAMD17-98-D-022

July 1998

EXECUTIVE SUMMARY

This Environmental Assessment (EA) was prepared in accordance with guidance provided in Army Regulation (AR) 200-2, *Environmental Effects of Army Actions*, dated December 23, 1988, implementing the National Environmental Policy Act (NEPA) (42 U.S. Code [USC] 4321-4347). This EA, *Environmental Assessment of U.S. Army Medical Research and Materiel Command (USAMRMC)-Funded Research at Virginia Polytechnic Institute and State University (VPI&SU)*, was prepared by USAMRMC with assistance from Science Applications International Corporation (SAIC) under Contract Number DAMD17-98-D-022.

This EA describes and analyzes the potential adverse environmental impacts, including human health impacts, associated with conducting a proposed research project funded by USAMRMC. This analysis considers impacts expected from conducting the proposed research, cumulative impacts that might occur after several years, impacts resulting from association with other activities in the area, and impacts resulting from an accident or incident. The proposed research is viewed as a necessary component of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats such as brucellosis. There is currently no human brucellosis vaccine that is acceptable for use. VPI&SU scientists have been studying brucellosis vaccines for many years and have developed the vaccine currently endorsed by the U.S. Department of Agriculture as the official vaccine to prevent brucellosis in cattle. The proposed research – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* –submitted by VPI&SU to the USAMRMC in response to a Broad Agency Announcement solicitation will be conducted within existing VPI&SU facilities.

During the preparation of this EA, one alternative to the proposed action was identified. This alternative is to not conduct the research as proposed at VPI&SU (Alternative II, no action). This EA characterizes the reasonably predictable environmental impacts, including impacts to human health that might result from conducting either the proposed research at VPI&SU (Alternative I, the preferred alternative) or the alternative considered.

The principal conclusions of this EA are: (1) the conduct of the proposed research project– *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* (Alternative I, the preferred alternative) is not expected to result in significant adverse environmental impacts; (2) implementing the preferred alternative will likely result in important benefits to the U.S. by enhancing progress toward developing an acceptable vaccine against human brucellosis; and (3) ceasing the proposed research project (Alternative II, no action) will eliminate the negligible environmental impacts associated with conducting the research, but it will also eliminate potentially significant advances in brucellosis vaccine development.

TABLE OF CONTENTS

1.0 PURPOSE AND NEED FOR THE PROPOSED ACTION

This EA describes and analyzes the potential adverse environmental impacts, including human health impacts, associated with conducting a proposed research project funded by the USAMRMC. The proposed research was submitted to the USAMRMC in response to a Broad Agency Announcement solicitation. The proposed research – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* – will be conducted at VPI&SU and is described in Section 2.0. This analysis considers impacts expected from conducting the proposed research, cumulative impacts that might occur after several years, impacts resulting from association with other activities in the area, and impacts resulting from an accident or incident. One alternative to the proposed action is also discussed (see Sections 3 and 5).

The proposed research study is viewed as a necessary component of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats. The USAMRMC was established in 1994 as a major subordinate command of the U.S. Army Medical Command (MEDCOM). The USAMRMC is the lead agent for the Department of Defense (DoD) Biological Defense Research Program (BDRP). Research and development activities in support of the BDRP are conducted at military research facilities and through contracts and Cooperative Research and Development Agreements with universities, other institutions, and industry. These programs are directed and monitored by USAMRMC headquarters staff officers from grant award through completion.

The bacterium *Brucella*, the causative agent of brucellosis, has been identified as a potential biological warfare threat. Currently, there is no effective, acceptable vaccine for human brucellosis. The proposed research is part of an overall effort to develop a vaccine that will protect troops against debilitating, antibiotic-resistant brucellosis. The objective of the proposed research project is to develop a system for generating *Brucella* antigens using recombinant deoxyribonucleic acid (DNA) molecules to generate an immune response in mice that provides protective immunity against challenge by a disease-causing strain (see Section 2.3). Research mice will be immunized with the vaccinia virus/*Brucella* recombinants and then challenged with virulent *Brucella* to determine the effectiveness in preventing infection.

NEPA (42 U.S. Code [USC] 4321-4347) requires that each Federal agency consider the potential environmental impacts associated with proposed major actions. The Council on Environmental Quality (CEQ), Executive Office of the President, has promulgated regulations implementing NEPA (40 Code of Federal Regulations [CFR] Parts 1500-1508). AR 200-2, *Environmental Effects of Army Actions*, dated December 23, 1988 (32 CFR 651), is the Department of the Army's (DA) implementation of NEPA and the CEQ regulations. USAMRMC environmental policy requires that an EA be prepared in accordance with AR 200-2 and CEQ regulations for proposed actions involving the operation of biosafety level (BL)-3/BL-4 laboratories. This EA was prepared in accordance with AR 200-2 and CEQ regulations.

Programmatic aspects of the BDRP were previously evaluated within the context of NEPA. The BDRP Final Programmatic Environmental Impact Statement (FPEIS) was prepared by the DoD in 1989 to examine the possible and probable environmental impacts of BDRP activities. The Record of Decision (ROD) resulting from the BDRP FPEIS found that certain aspects of the program were controversial (e.g., aerosol testing, genetically engineered microorganisms [GEMs]). However, the analysis found no evidence of major negative environmental impacts. Various public and government groups were involved with preparing the BDRP FPEIS. Dialogues and analyses indicated that public concerns were programmatic in nature and not directly related to specific sites within the BDRP. The analyses found that any potential adverse environmental impacts to the human environment associated with the continuation of BDRP research efforts were minimal. In this EA, BDRP activities, funded by the USAMRMC and performed at VPI&SU, are examined for their potential to cause significant adverse environmental impacts.

2.0 DESCRIPTION OF THE PROPOSED ACTION

2.1 INTRODUCTION

The bacterium *Brucella* is the causative agent of human brucellosis, also referred to as Bang's disease, undulant fever, Malta fever, or Mediterranean fever. Brucellosis in humans is caused by one of five *Brucella* species and its distribution is worldwide. *Brucella* also causes disease in animals and is transmissible from animals to humans. Brucellosis is rare in the U.S. because food sanitation and effective animal brucellosis control programs prevent its occurrence and transmission. Researchers at the VPI&SU are responsible for developing the animal vaccine currently endorsed by the U.S. Department of Agriculture (USDA) as the official vaccine for preventing brucellosis in cattle. Although a brucellosis vaccine is available for animals, there is currently no acceptable (safe and effective) vaccine for humans. Treatment for infected humans involves administering antibiotics immediately following suspected exposure. Symptoms of brucellosis may include fever, chills, night sweats, headache, muscle and joint pain, and profound fatigue. Disease symptoms may become evident within days of exposure or may develop gradually. Untreated, brucellosis rarely causes death, but it may result in chronic debilitating symptoms.

Humans acquire brucellosis by consuming *Brucella*-contaminated animal products or by coming into contact with diseased animals or their secretions. In rare cases, brucellosis may be transmitted from close person-to-person contact. *Brucella* is highly infectious, and human brucellosis can result from exposure to as few as 10 *Brucella* organisms (Harding and Liberman, 1995; Kaufmann, 1995; Kaufmann and Boyce, 1995). In fact, brucellosis has been a commonly reported laboratory-acquired infection with exposures occurring from entry of the organism through microscopic breaks in the skin, accidental sticks with contaminated objects, inhaling organisms from contaminated air, direct contact of contaminated materials with mucous membranes of the nose or eye, or accidental ingestion (Centers for Disease Control and Prevention / National Institutes of Health [CDC/NIH], 1993; Harding and Liberman, 1995; Sewell, 1995).

In October 1996, the VPI&SU Office of Sponsored Programs submitted a research proposal to USAMRMC in response to USAMRMC Broad Agency Announcement 95-1. The proposed study, *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* (USAMRMC Log Number 96172001), was detailed in this research proposal (VPI&SU, 1996). This proposed study is a continuation of brucellosis vaccine research efforts (human and animal) by scientists at the VPI&SU Virginia-Maryland Regional College of Veterinary Medicine, Center for Molecular Medicine and Infectious Diseases (CMMID), Infectious Disease Unit (IDU). Research regarding *Brucella* has been conducted at VPI&SU since 1978.

The proposed research is described in Section 2.3. As with other potentially disease-causing microorganisms, work with *Brucella* requires the application of special work practices and engineering controls to ensure worker and public safety as well as the integrity of research findings. Safety practices and procedures are discussed in Section 2.4.

2.2 ORGANIZATION, LOCATION, AND FACILITIES

Founded in 1872, VPI&SU, also known as Virginia Tech, is the largest university in Virginia. VPI&SU operates 100 buildings, a Corporate Research Center, and an airport, on 2,600 acres in Blacksburg, Virginia. VPI&SU also maintains a 1,700-acre research farm in Montgomery County, Virginia. Scientists within the VPI&SU Virginia-Maryland Regional College of Veterinary Medicine, CMMID, will conduct the proposed research project.

An estimated 3,500 funded research projects ($143 million) are in progress at the VPI&SU. Over the past 10 years, the Virginia-Maryland Regional College of Veterinary Medicine has received over $1,000,000 to conduct brucellosis research from various sources, including the USDA and the DA.

The proposed research will be conducted in Buildings 146B and 447, located on Price's Fork Road on VPI&SU main campus. Constructed in 1995, Building 146B is about 7,600 square feet in size and houses the specialized engineering controls necessary for conducting work with an organism such as *Brucella* (see Section 2.4.1). Building 447 was constructed for the School of Veterinary Medicine in 1954 and is 2,236 square feet in size. The animals required for the proposed study will be housed in Building 447 throughout the course of the study, pending the implementation of plans to consolidate all animal care and housing associated with the proposed action in Building 146B.

2.3 PROPOSED STUDY ACTIVITIES

For a detailed description of proposed study techniques and activities, see Appendix A.

The purpose of the proposed research is to develop a vaccine to prevent brucellosis in soldiers exposed to antibiotic-resistant *Brucella* species. There is currently no acceptable vaccine for preventing brucellosis in humans and the method of preventing disease in exposed individuals is treatment with antibiotic(s). If an individual were exposed to a genetically altered, antibiotic-resistant form of *Brucella*, effective antibiotics might not be available to prevent disease. The proposed research involves using genetic engineering techniques to produce recombinant DNA molecules containing DNA from vaccinia virus strains and from *Brucella* species. Researchers will systematically evaluate the recombinants for potential use in developing a vaccine that will be effective whether or not a *Brucella* strain has been intentionally altered for antibiotic resistance.

The function of a vaccine is to cause a primary immune response against a relatively harmless form of an antigen (foreign material such as bacteria or viruses) so that when that antigen is encountered again in a more harmful form, the body will produce a more effective response. Vaccination induces the immune system to develop a memory for a particular antigen, enabling the system to react quickly and more extensively upon subsequent infection. The process of vaccine development requires discovering which antigenic components of a pathogen (e.g., *Brucella*) will best stimulate an effective immune response. *Brucella* affects several cell types within the body, and lives within them as an intracellular pathogen. Once *Brucella* has infected a cell it becomes more difficult for the body to recognize and respond to it. Therefore, a successful *Brucella* vaccine candidate must be capable of stimulating the immune system to recognize it both before and after it infects cells. A humoral immune response is one involving the production of antibodies (proteins specific to antigens) that circulate in the blood. A cell-mediated immune response involves the recognition and destruction of infected cells. Researchers at VPI&SU will focus on developing a vaccine that will produce a strong cell-mediated immune response because *Brucella* is an intracellular pathogen. It is expected that a successful vaccine candidate will also produce a humoral immune response. The *Brucella* antigens required to achieve this protective cell-mediated immunity have not been defined as yet; therefore, *Brucella* antigens will be studied individually and in combination for their effectiveness.

Vaccinia virus has been selected as the biological vehicle to introduce and express genes in the cells that present *Brucella* antigens to the immune system. Vaccinia virus was selected as the vector for *Brucella* genes because it replicates intracellularly; it has been widely and safely used in humans in the smallpox vaccine; and a variety of *Brucella* proteins can be produced by vaccinia virus recombinants. The research will involve producing genetically engineered vaccinia virus/*Brucella DNA* recombinants that express various *Brucella* antigens and then comparing their ability to produce immune responses in mice. Two strains of vaccinia virus with potential for use as vaccines will be used. One strain is known to allow the

safe vaccination of humans with immune system deficiencies and both strains are known to induce immune responses in mice.

The ability of various recombinants to produce immunity in mice will be studied over a 2-year period. To compare immune responses, vaccinia virus/*Brucella* recombinants expressing one or more *Brucella* antigens will be constructed. The BALB/c mouse model will be used to test each recombinant for its ability to induce protective immunity. It is anticipated that 20 BALB/c mice will be required to test each recombinant. An estimated total of 960 mice (including controls) will be used. Groups of five mice will be vaccinated with low and high doses of each vaccinia virus/*Brucella* recombinant vaccine and with controls. Some mice may be revaccinated. The mice will be challenged with virulent (disease producing) *Brucella abortus* or *Brucella melitensis* strains at 6 to 7 weeks after vaccination. At specified intervals, humoral and cell-mediated immune responses to *Brucella* antigens will be analyzed by examining mouse spleens and blood. The mice will be sacrificed 2 weeks after the challenge, at which time additional blood samples will be analyzed.

The proposed study will require eight personnel; two full-time (one postdoctoral fellow; one technician) and six part-time workers (four faculty members and two graduate students) (Boyle, 1998a). It is anticipated that seven people will be working with vaccinia virus (two full-time and five part-time) and eight with *Brucella* (two full-time and six part-time). There are 14 certified users of the biosafety level 3 (BL-3) suite (Boyle, 1998a).

2.4 SAFETY

The proposed research requires the use of materials that require special handling to mitigate potential risks to human health and the environment. These materials include *Brucella abortus*, *Brucella melitensis*, and vaccinia virus. In addition, the proposed research involves the use of recombinant DNA molecules, chemicals, and radioisotopes. Prior to awarding funding for this research, the VPI&SU Manager of Occupational Health and Industrial Hygiene of the Environmental, Health, and Safety Services (EHSS) submitted a Facility Safety Plan to the USAMRMC detailing existing VPI&SU safety and occupational health programs under which the proposed research will be performed (Young, 1996).

The USAMRMC Safety Officer reviewed and approved the VPI&SU Facility Safety Plan (Hawley, 199. VPI&SU laboratory operations must adhere to the written safety and health programs prepared and administered by the EHSS as well as the written procedures developed specifically for the proposed research. These programs incorporate applicable law and regulations and include:

Laboratory Safety..... .....*Chemical Hygiene Plan*
Hazardous Materials Safety.....*University Hazardous Waste Contingency Plan*
Radiation Safety..... .....*Radiation Safety Handbook*
Biological Safety..... .....*Bloodborne Pathogen Exposure Control Plan*
....*Biotechnology Oversight Committee Charter*
....*IDU Operations Manual*
Occupational Health..... .....*Occupational Health Assurance Program*

2.4.1 Biological Safety

Both the VPI&SU and the DA require adherence to the biological safety guidelines described in *Biosafety in Microbiological and Biomedical Laboratories* (CDC/NIH, 1993). These guidelines recommend the laboratory practices, techniques, facilities, and equipment necessary to contain infectious organisms of varying degrees of pathogenicity and virulence and their products. These measures have been developed to minimize risks to human health and the environment. Regardless of location, research funded by the DA and involving biological defense agents such as *Brucella* must also meet the safety requirements

detailed in 32 CFR Parts 626 (*BDRP Safety Program*, AR 385-69) and 627 (*Biological Defense Safety Program Technical Safety Requirements*, DA Pamphlet 385-69). These regulations require implementing the CDC/NIH *Guidelines on Biosafety in Microbiological and Biomedical Laboratories*.

The guidelines describe the four biosafety levels (BLs) established by the CDC and NIH for conducting laboratory operations with infectious agents and/or their toxins. BL-1 practices, safety equipment, and facilities are appropriate for facilities in which work involves defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. BL-2 practices, safety equipment, and facilities are appropriate for facilities in which work involves the broad spectrum of indigenous (native) moderate-risk agents present in the community and associated with human disease of varying severity. Work with indigenous or exotic agents that have serious or lethal consequences if inhaled requires BL-3 containment. BL-4 practices, safety equipment, and facilities are required for work with dangerous and exotic agents posing a high individual risk of life-threatening disease. The CDC/NIH guidelines include "agent summary statements" that provide specific information on laboratory hazards associated with various agents and guidance for selecting appropriate BLs. Under the CDC/NIH guidelines, the laboratory director is responsible for determining the appropriate BL based upon "the virulence, pathogenicity, biological stability, route of spread, and communicability of the agent; the nature or function of the laboratory; the procedures and manipulations involving the agent; the endemicity of the agent; and the availability of effective vaccines or therapeutic measures (CDC/NIH, 1993)."

It has been determined that the proposed research requires the use of BL-2 practices for work with vaccinia virus and BL-3 practices for work involving *Brucella* (Young, 1996). BL-3 "differs from BL-2 in that (1) more extensive training in handling pathogenic and potentially lethal agents is necessary for laboratory personnel; (2) all procedures involving the manipulation of infectious material are conducted within biological safety cabinets, other physical containment devices, or by personnel wearing appropriate personal protective clothing devices; [and] (3) the laboratory has special engineering and design features, including access zones, sealed penetrations, and directional airflow (32 CFR 627)."

The VPI&SU BL-3 facilities in which work with *Brucella* is conducted are located in the IDU (Building 146B) of the CMMID. Laboratory work involving animal challenges, *Brucella* cultivation, and DNA extractions are conducted in the BL-3 suite. This facility was constructed in 1995 specifically for research involving microorganisms requiring BL-3 containment. Standard operating procedures for work conducted in the BL-3 laboratory at VPI&SU are found in the *IDU Operations Manual*.

BL-3 facilities such as those in which the proposed research will be conducted must have signs posted on all doors indicating their BL-3 designation, agent(s) in use within, and individuals to contact in case of an emergency. Measures to limit and control access to BL-3 laboratories are required. At VPI&SU, access to the BL-3 laboratory is restricted to personnel directly involved with the work and certified for entry. The principal investigator confers certification after training. The BL-3 laboratory is locked at all times. Two doors allow access to the laboratory; these doors are magnetically controlled to impede opening at the same time which would disrupt the required air pressure balance. The BL-3 laboratory operates under negative pressure to the outside, which results in a net flow of air into the facility. Two separate motors control airflow. Heating, ventilation, and cooling equipment in the BL-3 suite are controlled by a computer-based system. The control room for this system is located in the Physical Plant Department in the Maintenance Building. Air entering the BL-3 suite is filtered, and in accordance with CDC/NIH guidelines, outgoing air from BL cabinets is filtered through high-efficiency particulate air (HEPA) filters, and laboratory air is exhausted to a stack on the roof. Entry into BL-3 areas is through an adjacent hallway. To maintain the required directional airflow, magnetic controls ensure that laboratory doors cannot be opened unless the adjacent outer hallway door is closed. Personnel don gloves and gowns upon entering the BL-3 facility. Head coverings and masks are required when activities involve the potential for generating aerosolized microorganisms (e.g., pipetting or centrifugation). Surfaces within laboratories

and adjacent hallways are sealed with epoxy paint and all penetration to the room sealed with silicone or other approved sealant. Electrical outlets and switches must be the kinds that reduce the potential for contamination. Potentially contaminated work materials are not removed from the BL-3 facility until they are rendered noninfectious by chemical disinfection or autoclaving. To limit moving potentially contaminated materials, autoclaves are positioned within hallway areas connecting to outer "clean" areas. The permissible flow of people, equipment, animals, and experimental materials within the IDU is detailed in the *IDU Operations Manual*. The restrictions on the movement of these entities are designed to protect worker health and safety, prevent cross-contamination in adjacent areas, and prevent the breech of containment. Any changes in traffic pattern guidelines must be posted in writing. Drains contain disinfectant traps that are changed either after use or weekly. Alarms sound in case of a power failure or if the air-handling system shuts down. Procedures for the actions required during a power failure are detailed in the *IDU Operations Manual*.

There are two biological safety cabinets in the BL-3 suite in which work with biohazardous agents is performed. Emergency equipment, including fire extinguishers, emergency shower, and eyewash stations, is located within the BL-3 suite. A sprinkler system for fire suppression is installed in the ceiling. An adjacent BL-2 suite contains personal protective equipment such as Tyvek® coveralls and respirators for use in case of an emergency in the BL-3 suite.

In addition to safety requirements related to the use of *Brucella*, the proposed research also requires adherence to standards and procedures for the safe use of recombinant DNA molecules. Recombinant DNA molecules are defined as either "molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell" or "molecules that result from the replication of those described above." The *Guidelines for Research Involving Recombinant DNA Molecules* (NIH, 1997) establish guidelines for work involving recombinant DNA molecules. The NIH guidelines specify practices for constructing and handling recombinant DNA molecules and the organisms or viruses containing recombinant DNA molecules. The guidelines specify procedures for authorizing and overseeing such work, laboratory facilities, and work practice controls. In addition, the guidelines classify agents according to risk and establish procedures for institutional oversight (NIH, 1997).

Brucella is classified by the NIH guidelines as a Class 3 agent. According to the NIH guidelines, work involving recombinant DNA procedures on Class 3 agents must be registered with the Institutional Biosafety Committee (IBC). At VPI&SU, the Biotechnology Oversight Committee (BOC) has subsumed the IBC. The BOC publishes written standards for conducting research involving recombinant DNA and reviews new VPI&SU research involving recombinant DNA. The BOC reviews projects and grants written approval before work is initiated (Young, 1996).

VPI&SU has applied for registration with the CDC tracking system, in accordance with 42 CFR 72, *Additional Requirements for Facilities Transferring or Receiving Select Agents*. Inventories of virulent stocks are maintained in a notebook. An inspection of VPI&SU BL-2 and BL-3 laboratories by the USAMRMC biosafety officer was conducted on September 24, 1997 in accordance with AR 385-69 (32 CFR Parts 626 and 627, *U.S. Army Biological Safety Program*). The BL-2 and BL-3 laboratories, animal facilities, and support facilities were inspected using the Basic Checklist for Biosafety Levels 1, 2, and 3 (DA Pamphlet 385-69, 32 CFR Part 627). The VPI&SU facilities inspected were found to meet or exceed physical standards for BLs 1, 2, and 3 as described in the CDC/NIH guidelines and DA Pamphlet 385-69. Operational procedures observed or discussed were also in accordance with applicable regulations. The USAMRMC biosafety officer recommended that VPI&SU conduct monthly BL-3 safety inspections and quarterly BL-2 safety inspections in accordance with 32 CFR Part 626 (Hawley, 1997).

2.4.2 Chemical Safety

Hazardous chemicals that will be used in the conduct of the proposed research include phenol and chloroform in small quantities (Young, 1996). The handling and use of hazardous chemicals is regulated by Occupational Safety and Health Administration (OSHA) regulations. The Manager of Occupational Health and Laboratory Safety Programs has prepared the VPI&SU Chemical Hygiene Plan (CHP) in accordance with OSHA regulations (29 CFR 1910.1450, *Occupational Exposure to Chemicals in Laboratories*) and oversees its implementation. University-wide policies and procedures for the safe handling and use of chemicals are contained in the VPI&SU CHP as required by OSHA regulations. The University Laboratory Safety Committee monitors compliance with the CHP. Principal investigators must appoint Laboratory Chemical Hygiene Officers. Laboratory-specific procedures must be developed, put into a written plan, and approved by the Principal Investigator. OSHA regulations require training for all personnel prior to work assignments or new tasks with the potential for exposure to hazardous chemicals. Information and training continue through occasional refresher courses. Training includes instructions for accessing Material Safety Data Sheets (MSDSs). The CHP and laboratory-specific procedures must provide information about handling controlled substances, chemical acquisition, chemical storage, potential health risks, environmental monitoring, personal protective equipment, use of fume hoods, safety procedures, and inspections and laboratory audits. In accordance with these regulations, VPI&SU has developed written safety policies and procedures for all university laboratory personnel. For information about chemical waste handling and disposal, see Section 2.6.

2.4.3 Radiologic Safety

Radioisotopes planned for use in the conduct of the proposed research include ^{3}H thymidine and ^{51}Cr. The U.S. Nuclear Regulatory Commission (NRC) regulates the use of radioisotopes. The proposed research will be performed under NRC license #45-09475-30 (expiration date September 30, 2003) (Smiley, 1998). NRC regulations require the preparation of written guidelines detailing the safe storage and handling of radiologic materials. The VPI&SU EHSS has published a Radiation Safety Handbook and administers University radiation safety policies through the Radiation Safety Office. The Radiation Safety Officer is on the staff of the EHSS and must be an individual with the skills and experience necessary to supervise all aspects of VPI&SU radiation measurement and protection activities as specified and required by the NRC. All new or unique procedures involving radioisotopes undergo hazard analyses by the Radiation Safety Officer. The VPI&SU Radiation Safety Committee (RSC) regulates the safe use of radioisotopes and radiation sources. The RSC approves Laboratory Authorities, the individuals charged with managing radioactive material use in a specified area. The procedures required for attaining authorization are detailed in the *Radiation Safety Handbook*, along with requirements for record keeping, general laboratory radiological safety, labeling, security, and facility requirements. Failure to adhere to requirements detailed for the safe handling and use of radioisotopes may result in disciplinary action. The NRC is empowered to revoke the license for safety violations.

2.5 SECURITY

The BL-3 laboratory and the building in which it is located are accessible only by key. The Faculty Supervisor to the IDU controls access to keys. This individual issues keys only to individuals who have been certified to work in the BL-3 laboratory (Boyle, 1998a).

2.6 WASTE STREAM MANAGEMENT

It is estimated that the proposed research will generate 800 liters (208 gallons) of liquid wastes and 80 kilograms (176 pounds) of solid wastes annually. Included in these estimated waste quantities are regulated wastes such as sharps (e.g., needles) and potentially contaminated materials, general solid

waste, and animal wastes. In accordance with CDC/NIH guidelines, all wastes contaminated or potentially contaminated with infectious material must be rendered noninfectious before disposal. This decontamination is accomplished by a combination of chemical and physical (autoclave) methods. Potentially contaminated liquid wastes must be decontaminated by treatment with a 3% Lysol® solution followed by sterilization by autoclave (90-minute cycle). Once rendered noninfectious by these methods, liquid wastes may be disposed of into the sanitary sewer system. Following decontamination, general solid waste may be disposed of without further treatment or special handling. Following decontamination, regulated medical wastes (e.g., animal wastes, culture material, and sharps) will be collected and removed by the EHSS for transfer to a contractor for off-site transport and incineration (Boyle, 1998a).

The quantity of hazardous waste generated from the proposed research is expected to be less than 10 liters annually (Boyle, 1998b). Noninfectious liquid wastes containing hazardous chemical wastes must be disposed of in accordance with VPI&SU rules and regulations pertaining to hazardous waste. VPI&SU hazardous waste procedures implement Virginia Department of Environmental Quality (VDEQ) Hazardous Waste Management Regulations promulgated under the Resource Conservation and Recovery Act (RCRA). The VPI&SU hazardous waste identification number is VAD074747908. In addition, the University has developed a Hazardous Waste Contingency Plan, which details procedures required for preventing or mitigating hazard to human health and the environment from accidents and incidents involving hazardous waste. This plan addresses actions required in case of hazardous materials spills, including emergency response procedures.

2.7 ANIMAL CARE AND USE

DA funding policy and VPI&SU guidelines require that all animals be housed, handled, and used in accordance with the Animal Welfare Act and the NIH *Guide for the Care and Use of Laboratory Animals*. Prior to designing the proposed study, it was determined that there were no alternatives to the use of live animals in which to assess effectiveness of immune responses induced by vaccinia virus/*Brucella* recombinants. It is anticipated that the proposed study will require 960 BALB/c female mice. The estimated number of animals required is based upon the number required for achieving statistically significant results. The selection of BALB/c mice (an inbred strain) as a model allows the use of fewer mice than might be required with other strains.

The mice used in the proposed study will be housed in either the IDU animal quarters or Building 447, which is adjacent to Building 146B. Throughout the course of the investigation, mice will be housed in micro-isolator units that meet BL-3 specifications. Mice will be transported a distance of approximately 100 yards in micro-isolator cages to Building 146B by animal care workers and returned to Building 447 for care after use (Boyle, 1998c). Cage cards identify experimental animals, the treatment they are undergoing, and related biohazards. Animal inventories are required daily, and laboratory animal care logbooks must be maintained. The building in which animals are maintained is kept locked at all times (Boyle, 1998a).

2.8 HUMAN HEALTH AND SAFETY

2.8.1 Worker Health and Safety

The VPI&SU EHSS Bloodborne Pathogen Exposure Control Plan details the protective measures required to ensure worker health and safety, including vaccination requirements and medical monitoring recommendations. There is no vaccine for *Brucella* available for use in humans. The EHSS Bloodborne Pathogen Exposure Control Program directs medical monitoring for personnel working with *Brucella* at the VPI&SU. Medical monitoring is a requirement of both the CDC/NIH guidelines and AR 385-69. In

accordance with CDC/NIH guidelines and AR 385-69, baseline serum samples (blood samples obtained before working with *Brucella*) are obtained from workers. CDC/NIH guidelines also recommend, and AR 385-69 requires, that additional periodic blood studies be conducted for those working with *Brucella*. Currently, this periodic monitoring is not performed, however, the Principal Investigator has requested that the University arrange for implementing this periodic medical monitoring for individuals working with *Brucella*.

Maintenance workers are protected from exposure to potential pathogens by limiting access to the BL-3 facilities. Maintenance workers are only permitted onto the premises during routine maintenance, which occurs every 3 months. During routine maintenance, all BL-3 work ceases. In the event that emergency repairs are required, maintenance personnel enter only after all BL-3 work is shut down and stored and animals are removed from the work areas (Boyle, 1998a).

VPI&SU requires that all persons working directly with vaccinia virus must be vaccinated before working with the virus (Boyle, 1998a). Prior to vaccination, workers must be informed of possible adverse reactions to the vaccination. Workers unable to undergo vaccination for medical reasons are not permitted to work with vaccinia virus (Young, 1996).

The EHSS Occupational Health Assurance Program provides respiratory protection services. Individuals whose duties may require the use of respirator must be included in the VPI&SU respiratory protection program and must receive training, undergo pulmonary function studies and evaluation by a physician, and be fit-tested for respirator use on a regular basis.

2.8.2 Public Health and Safety

The proposed research does not involve the use of human research subjects. When a vaccine for human use is developed from this research, its advanced development, testing, production, and use in humans will be regulated by the U.S. Food and Drug Administration (FDA). In accordance with FDA regulations, any vaccine developed will be evaluated within the context of NEPA before licensure (DA, 1996).

2.8.3 Accidents and Incidents

In the conduct of work with *Brucella* at VPI&SU since 1978, there have been incidences of accidental inoculation with *Brucella*. Prophylactic antibiotics were administered within 72 hours to the individuals reporting potential exposure and no cases of brucellosis resulted. No individuals have become seropositive since 1978 (Boyle, 1998a; Schurig, 1998).

In accordance with AR 385-69, VPI&SU coordinates emergency preparedness with local emergency service providers and maintains formalized agreements with them. In the event of a medical emergency, injured personnel would be transported by the VPI&SU rescue squad to the nearby Montgomery Regional Hospital in Blacksburg, Virginia. Additional medical facility support is available in Roanoke or Charlottesville, Virginia.

Work with vaccinia virus has been conducted at VPI&SU since 1994 without accident or incident (Boyle, 1998a). According to NIH guidelines, spills or accidents resulting in actual or potential exposures to recombinant DNA molecules must be reported to the EHSS, the BOC, and the NIH Office of Recombinant DNA activities.

3.0 ALTERNATIVES CONSIDERED

3.1 INTRODUCTION

The proposed action and subject of this EA is a research project funded by the USAMRMC – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* (Alternative I, the preferred alternative). During the preparation of this EA, one alternative to the proposed action was identified. This alternative involves discontinuing plans to conduct the proposed research (Alternative II, no action).

3.2 ALTERNATIVE I – CONDUCT PROPOSED RESEARCH AT VPI&SU

Alternative I entails the activities necessary to conduct the currently planned research study – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* – as proposed to USAMRMC by VPI&SU. This alternative is preferred because the proposed research activities are likely to produce important information and increased understanding of methods to prevent human brucellosis, a disease caused by *Brucella*, a potential biological warfare threat. The proposed project is the product of VPI&SU investigators, is related to ongoing research at VPI&SU involving immunity to *Brucella*, and as such is not transferable to another site or research group. Alternative I is preferred as the option that better meets national defense needs.

3.3 ALTERNATIVE II – NO ACTION

Alternative II entails discontinuing USAMRMC plans to fund the proposed study at VPI&SU. This alternative is not preferred, because of the need to maintain continuing research efforts toward developing a safe and effective vaccine against human brucellosis. The proposed research project has been critically reviewed by USAMRMC and determined to have the potential to advance brucellosis vaccine research. Alternative II is not preferred, because it would impair national defense by disrupting research efforts directed toward protecting U.S. soldiers from brucellosis, a potential biological warfare threat.

4.0 AFFECTED ENVIRONMENT

4.1 INTRODUCTION

This section of the EA describes aspects of the biophysical and socioeconomic environment that could potentially be impacted by the proposed action.

4.2 LAND USE AND GEOLOGY

The VPI&SU main campus is located on 2,600 acres in the town of Blacksburg, Virginia. The proposed biomedical research will be conducted in the VPI&SU Building 447 and the IDU in Building 146B. The buildings are located at the Veterinary Research Center at the CMMID (Buildings 440 and 440A) on Prices Fork Road (Master Building List for Virginia Tech, 1998). Two 70-square foot laboratories in the IDU will be used during the proposed research.

The town of Blacksburg is situated on 18.8 square miles in Montgomery County, which covers 395 square miles in southwestern Virginia. Montgomery County lies between the Appalachian Plateau to the west and the Blue Ridge Mountains to the east. The town of Blacksburg and VPI&SU are located between the New and Roanoke Rivers at an elevation of 2,000 feet. Price Mountain is located approximately 2 miles southwest of the VPI&SU campus, and Jefferson National Forest lies to the west (U.S. Geological Survey [USGS], 1983).

Montgomery County is located in the Blue Ridge and Ridge and Valley physiographic provinces. The New River drainage to the Gulf of Mexico and the Roanoke River drainage to the Atlantic Ocean are separated by a divide that crosses the county from north to south (Soil Conservation Service, 1985).

The soils in the Blacksburg area are about 21% Groseclose, 15% Poplimento, 15% Duffield, and 55% minor soils. The soils underlying Buildings 447 and 146B are of the Groseclose-Urban land complex. These soils are gently sloping, on 2 to 7% slopes, and are located on broad ridgetops. Groseclose soils have a loam or silt loam surface layer that may be cherty, and clay subsoil. They are deep and well drained. The soils formed in limestone, shale, and sandstone residuum and colluvium. Undisturbed Groseclose soils have slow permeability, moderate available water capacity, and moderate potential frost action. The shrink-swell potential is high. Groseclose soils have low organic matter content and natural fertility. In disturbed areas, the soil characteristics are extremely variable. The erosion hazard is moderate. The Groseclose soil profile is typically a 10-inch thick brown loam surface layer and subsoil is about 29 inches thick. The subsoil is sticky and plastic clay, and is yellowish brown from 10 to 28 inches in depth. At depths of 28 to 39 inches, the subsoil is mottled in brown, yellow, and red. The substratum is also mottled brown, yellow, and red between depths of 39 and 72 inches. From 39 to 51 inches in depth, the substratum is sticky and plastic clay. Clay loam is at present between 51 and 72 inches. The soil complex consists of about 50% Groseclose soils, 20% urban land, and 30% other soils. Buildings, structures, roads, and parking lots cover urban land (Soil Conservation Service, 1985).

4.3 CLIMATE AND AIR QUALITY

Montgomery County has a moderate continental climate. The annual precipitation in Blacksburg is 40.91 inches. Annual snowfall in the area is 26.4 inches. Prevailing winds are from the west. The average high and low temperatures range from 82.3ºF to 19.0ºF. The highest temperatures occur during July and the lowest occur during January. Mean monthly temperatures range from 70.6ºF in summer to 29.6ºF in winter (National Climatic Data Center [NCDC], 1990).

The VDEQ is responsible for monitoring air quality in accordance with the National Ambient Air Quality Standards (NAAQS) (VDEQ, 1998a). The Blacksburg area is in attainment for all NAAQS, including carbon monoxide, sulfur dioxide, nitrogen dioxide, ozone, lead, and particulate matter less than 10 microns in diameter (PM10) (VDEQ, 1998b).

4.4 WATER RESOURCES AND WETLANDS

The town of Blacksburg and VPI&SU lie within the boundaries of the Upper Roanoke watershed. The watershed is classified as having less serious water quality problems and as being highly vulnerable to stressors such as pollutant loadings. In 1996, 80%-100% of the assessed rivers and lakes in the Upper Roanoke watershed met U.S. Environmental Protection Agency (EPA) drinking water criteria (USEPA, 1998b). Groundwater availability in Montgomery County is highly variable, and groundwater quality is described as generally good. The land on which Buildings 447 and 146B is located drains into Stroubles Creek (USGS, 1983).

The Virginia Department of Health regulates water systems in the state (USEPA, 1998a). Drinking water for the town of Blacksburg and VPI&SU is obtained from the Blacksburg-Christiansburg-VPI&SU Water Authority, which pumps water from the New River in southwestern Virginia. Water meter readings for 1997 for the VPI&SU campus totaled 405,783,227 gallons. Buildings 447 and 146B are not individually metered (Roschelli, 1998).

The wetland nearest Building 447 on the VPI&SU campus is less than .5 mile away. The wetland is palustrine with an unconsolidated bottom, impounded, and permanently flooded (Fish and Wildlife Service, 1990).

4.5 PLANT AND ANIMAL ECOLOGY

The Virginia Department of Game and Inland Fisheries reports no currently documented threatened or endangered species in the area around the research facility at VPI&SU (Reay, 1998).

4.6 HISTORICAL AND CULTURAL RESOURCES

The VPI&SU was founded as a land-grant college in 1872 and is today the largest university in Virginia. The campus includes nearly 100 buildings, an airport, stadium, coliseum, and several thousand acres of agricultural research land. The campus includes the restored home of a revolutionary war hero, and the birthplace of two Virginia governors. The proposed action will be conducted in Buildings 146B and 447 located on VPI&SU main campus (see Section 2.2). Building 146B was constructed in 1995 and Building 447 was constructed in 1954.

4.7 ENERGY RESOURCES

Virginia Tech Electric Services (VTES) supplied 373,248 kilowatts to the IDU building (Building 146B) between April 1997 and March 1998 (VTES, 1998). Building 146B was heated with 3,741,400 cubic feet of natural gas from United Cities. The Virginia Tech Central Plant supplies steam heat to Building 447. For the 1996-1997 year, Building 447 used an estimated 0.113% of the plant's total steam production. Building 447 has a capacity of 341,000 British Thermal Units (BTUs) of heat installed (Roschelli, 1998). Specific energy usage for the proposed research project facilities is not available because the specific facilities are not individually metered. However, the total square footage of the two laboratories in the IDU is 1,540 square feet, which is a small fraction of VPI&SU energy resource requirements.

4.8 SOCIOECONOMIC ENVIRONMENT

The population of Montgomery County was 76,800 in 1996, an increase of 13,284 from 1980. Between 1980 and 1996, the population of the town of Blacksburg grew from 30,638 to 36,400. In 1990, the population of Blacksburg was 87.4% white, 7.7% Asian or Pacific Islander, 4.3% black, 1.8% Hispanic, and less than 1% American Indian, Eskimo, or Aleut. Of those age 25 years or older, 61.6% had earned at least a bachelor's degree. VPI&SU has a total annual budget of approximately $491 million and conducts $143.8 million of research annually. In 1997 on-campus enrollment was about 25,000 students. VPI&SU employs approximately 1,425 full-time faculty in addition to researchers, administrators, and staff (Virginia Tech web site, 1998).

In 1989, 12.8% of families and 37% of all persons living in Blacksburg had incomes below the poverty level (University of Virginia Library Social Services Data Center County and City Data Books, 1998).

4.9 TRANSPORTATION

The town of Blacksburg and VPI&SU are located approximately 40 miles southwest of Roanoke, Virginia. VPI&SU is accessible by automobile from I-81, U.S. 11, and U.S. 460. I-81 runs southeast-northwest south of VPI&SU. Route 11 runs east-west. Both I-81 and Route 11 intersect U.S. 460 West south of Blacksburg. The U.S. 460 Bypass intersects Prices Fork Road west of Building 447 and U.S. 460 passes through the business district east of VPI&SU.

Access to VPI&SU is provided by the Blacksburg Transit Town-wide Service. Greyhound Bus Lines serve the town of Blacksburg. Domestic airlines use Roanoke Regional Airport in Roanoke, and the Virginia Tech Airport is available to private aircraft.

5.0 ENVIRONMENTAL CONSEQUENCES

5.1 INTRODUCTION

In this section, the potential for significant environmental impacts (direct, indirect, and cumulative) likely to result from the proposed *Brucella* research at VPI&SU will be discussed. This discussion will identify cause and effect relationships between the proposed action and impacts to the environment, including examining impacts that may not necessarily occur but that are reasonably predictable. The term "consequence" refers to the outcome of an event or events without considering probability. Where possible, potential events will be characterized in terms of both their potential consequence and the probability (likeliness) that they will occur.

5.2 ENVIRONMENTAL CONSEQUENCES

5.2.1 Land Use and Geology

It is highly unlikely that the proposed research project (Alternative I) would impact land-use patterns, geology, or soils at VPI&SU, or within Blacksburg, Virginia. All proposed activities will be conducted in existing facilities that have been sited in conformance to local topography. It is estimated that the quantity of wastes generated from the proposed research project will be negligible when compared to wastes generated from all of VPI&SU. The portion of wastes disposed of in local landfills will also likely be a negligible component of the total wastes from all of VPI&SU. Because construction is neither planned nor anticipated, no disruption of land-use patterns or geological resources is likely. Implementing Alternative II (no action) would eliminate any negligible impacts to land-use patterns, soils, or geological resources.

5.2.2 Climate and Air Quality

It is highly unlikely that negative impacts to air quality will result from the conduct of the proposed study (Alternative I). Impacts to air quality will result from incineration of regulated medical wastes and from on-road mobile sources of air pollution (trucks and automobiles) transporting employees and providing services in support of the proposed research. The contributions of these impacts to regional air quality are likely to be negligible in comparison to those of other activities in the area. Current regional air quality is good (see Section 4.3). Implementing Alternative II (no action) would eliminate the negligible impacts associated with conducting the proposed action.

5.2.3 Water Resources and Wetlands

Implementation of the proposed action is unlikely to significantly impact water resources near VPI&SU, or in the Blacksburg area. Quantitatively, wastewater contributions expected from conducting the proposed study are likely to be negligible in comparison with total wastewater discharges resulting from VPI&SU. In accordance with both Federal and state regulations, wastewater generated by all VPI&SU activities undergoes treatment at the Lower Stroubles Creek Wastewater Treatment Plant prior to discharge. Potentially contaminated wastewater generated in the BL-3 facility must be rendered noninfectious prior to discharge to the sanitary sewer system. Hazardous chemical waste, regulated medical waste, and radiologic waste generated by the proposed action must be segregated when generated. Adherence to Federal and state law and VPI&SU policy governing waste disposal further mitigates potential impacts to surface water resources. Implementing Alternative II (no action) would eliminate the negligible impacts associated with implementing the proposed action.

Adverse impacts to wetlands from implementing the proposed action (Alternative I) are highly unlikely. The proposed action will be conducted in existing facilities and no construction is planned or anticipated; therefore, stormwater runoff patterns will not be impacted. Wastewater will not be discharged to wetlands. Implementation of Alternative II (no action) would eliminate potential impacts associated with the proposed action.

5.2.4 Plant and Animal Ecology

It is highly unlikely that adverse impacts to plant or animal ecology will result from the conduct of the proposed study (Alternative I). No construction or renovation is planned that could impact plant or animal habitat. *Brucella* does not cause plant disease. Impacts to animals near the VPI&SU facilities in which *Brucella* research will be conducted are highly unlikely. The facilities in which mice will be housed have features that reduce the likelihood of animal escape. In the unlikely event that a mouse would escape during transport it would be unlikely to survive in the natural environment. Should a mouse escape and survive, its ability to transmit *Brucella* while alive is limited. Potential adverse impacts to susceptible native mammalian species would be possible from consumption of *Brucella*-infected mice. Careful transport and inventory of potentially infectious mice will mitigate these risks. Plans are currently under way to maintain all mice in Building 146B throughout the course of the study. This would eliminate the need to transport potentially infectious animals between buildings and mitigate the potential adverse impacts to animal ecology. Alternative II (no action) would eliminate any potential adverse impacts to local plant and animal ecology.

5.2.5 Historical and Cultural Resources

Adverse impacts to historic or archaeological resources are unlikely to result from implementation of the proposed alternatives. The proposed action will be conducted indoors in existing facilities that have been designed for their intended use. No renovations or construction are planned that would negatively impact unknown resources. Implementing Alternative II (no action) would eliminate any potential for adverse impacts on historical or archaeological resources.

5.2.6 Energy Resources

Adverse impacts to energy resources are unlikely to result from implementing the proposed action. The proposed research will be conducted in existing facilities in which similar activities are currently conducted. The proposed action is not anticipated to alter existing resource utilization. Implementing Alternative II (no action) would eliminate these negligible impacts on energy resources.

5.2.7 Socioeconomic Environment and Aesthetics

Implementation of the proposed action (Alternative I) will likely result in negligible positive impacts on the socioeconomic environment. An economic impact study conducted by VPI&SU estimates that activities of the Virginia-Maryland Regional College of Veterinary Medicine contribute significantly to the economy of Virginia. The estimated $48 million contribution was based on several factors, including the economic impact of research findings that improve and protect animal health. Significant impacts from noise or odors are not anticipated. Similar activities have been conducted at the proposed research facilities without observed impacts or complaints. It is unlikely that the proposed action will result in negative socioeconomic-environmental impacts. Implementing Alternative II (no action) would eliminate the minor positive impacts to the local economy likely to result from implementing the proposed action.

5.2.8 Transportation

Implementation of the proposed action (Alternative I) will likely have negligible impact on transportation resources. There is no construction or renovation planned that would alter existing traffic patterns. Commuting activities of 14 personnel involved in implementing the proposed action will likely have a negligible impact. Implementation of Alternative II (no action) will eliminate any potential for positive or negative impacts to transportation resources associated with the proposed action.

5.2.9 Public Opinion

Public opinion has been an issue in the conduct of biological warfare defense research and development activities and was extensively discussed in the BDRP FPEIS. There is strong congressional and public support for DoD policy of providing service men and women with the best possible protection against potential biological warfare agents. Potential criticisms, however, include the perceived potential for this research to be used for offensive purposes, the efficacy of biological defense vaccines, distrust of the military, and whether the military should be involved in vaccine development. Some public concerns relate to the existence of biological defense programs *per se*; others, to the intent, need for, and benefits of such programs. Some concerns are specific to the impacts of actions, such as the use of animals in the research and use and handling of recombinant DNA technology. Issues such as these are not unique to the proposed research but are concerns associated with vaccine and/or other biomedical research and development activities in general.

The government and facilities supported by the government (e.g., VPI&SU) do not engage in work related to the production or use of offensive biological weapons, as required by the *Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction* (the Biological Weapons Convention of 1972) to which the U.S. is a signatory.

The BDRP FPEIS examined the use of recombinant DNA technology and concluded that significant issues associated with its use were related to the existence of the biological defense program rather than to specific sites that were analyzed. The analysis performed in the BDRP FPEIS identified no actual significant adverse impacts resulting from the use of recombinant DNA technology. This conclusion was validated by subsequent biological defense site-specific assessments including the *U.S. Army Medical Research Institute of Chemical Defense EA* (USAMRICD, 1992), *Walter Reed Army Institute of Research EA* (WRAIR, 1993a), the *Walter Reed Army Institute of Research Leased Facilities EA* (WRAIR, 1993b), *BSL-2 Vaccine Facility at the Walter Reed Army Institute of Research at Forest Glen, Maryland EA* (WRAIR, 1994), and the *Joint Vaccine Acquisition Program Programmatic EA* (Joint Program Office for Biological Defense, 1997).

5.2.10 Human Health and Safety

The proposed research project at VPI&SU involves using *Brucella* species capable of causing human disease. Although rarely fatal, brucellosis can result in chronic adverse health effects if left untreated. The prompt administration of antibiotic therapy following known or suspected exposure is effective in preventing acute and/or chronic disease. Vaccinia virus presents virtually no risk to healthy humans, and low level risk for persons with weakened immune systems or who have a history of certain skin diseases. All persons working with vaccinia virus have been immunized (Boyle, 1998a).

5.2.10.1 Worker Health and Safety

The risk to workers of laboratory-acquired infections from the conduct of the proposed study (Alternative I) is minimized by implementing the environmental engineering and work practice controls described in the CDC/NIH guidelines (1993), AR 385-69, DA Pamphlet 385-69, and the *IDU Operations Manual*. Environmental engineering controls are in place in the VPI&SU BL-3 laboratory to prevent *Brucella* organisms from contaminating the laboratory environment. Risk of exposure is mitigated by the use of required laboratory work practices designed to reduce the likelihood of aerosol production during routine activities. Work practice controls used to prevent contamination of environments external to the BL-3 laboratory include disinfecting work surfaces, floors, and drains and segregating and autoclaving waste materials, work clothes, and other material prior to removal from containment facilities. In addition to the use of engineering and work practice controls to reduce the risk of exposure to *Brucella*, regular monitoring of worker health is required. Antibiotic therapy must be administered to workers with possible exposures. Prior to working with vaccinia virus, individuals are required to undergo vaccination and there have no incidences of illness (Boyle, 1998a). Significant impacts to worker health resulting from similar work have not been observed (DA, 1989). While there have been potential exposures to *Brucella* at VPI&SU, there have been no incidences of disease. Work with *Brucella* has been conducted at VPI&SU since 1978. Work with vaccinia virus has been conducted at VPI&SU since 1994 (Boyle, 1998a). Implementing Alternative II (no action) would eliminate the potential for adverse impact to worker health and safety associated with the conduct of the proposed study.

5.2.10.2 Public Health and Safety

The risk to public health from the conduct of *Brucella* research is negligible. Because of the redundant safety features required of BL-3 facilities, it is unlikely that the public would be exposed to viable *Brucella* originating from the VPI&SU laboratory. Adherence to Federal and state regulations pertaining to the safe handling and disposal of hazardous chemicals, radioisotopes, and potentially infectious material further mitigates the likelihood of impact to public health and safety. Similar work has been performed at VPI&SU without observed impacts to public health. For information pertaining to impacts resulting from an accident or incident, see Section 5.2.10.3. Implementing Alternative II (no action) would eliminate the potential for adverse impact to public health and safety associated with the conduct of the proposed study and the potential for positive impact to public health from developing a *Brucella* vaccine.

5.2.10.3 Accidents and Incidents

In accordance with requirements of AR 385-69, a maximum credible event (MCE) analysis has been developed for work with *Brucella* at VPI&SU. An MCE is a realistic worst-case scenario. The probability of such an accident occurring is remote when required procedures are used and there have been no such incidents associated with *Brucella* research at VPI&SU (Boyle, 1998a). In this scenario, a 1,000 milliliter (ml) culture containing one billion cfu per ml (1×10^9 cfu/ml) or 10^{12} cfu total of *Brucella melitensis* is spilled in the BL-3 laboratory. For determining the MCE, an infectious dose was assumed to be 10 organisms (Kaufmann, 1995; Kaufmann and Boyce, 1995). The spill therefore represents 1×10^{11} potential infectious doses. Of the liter of culture spilled, approximately 1% would become aerosolized ($0.01 \times 10^{11} = 10^9$ potential infectious doses). It was then assumed that of the 1% aerosolized, 90% would settle as droplets and 10% (0.9 ml) would remain aerosolized, resulting in 9×10^7 potential infectious doses ($0.01 \times 10^9 = 10^7$ potential infectious doses). Based upon air volume in the laboratory, it was then assumed that 9×10^5 potential infectious doses (approximately 1%) would reach the exhaust after 30 minutes and that 95% (8.5×10^5) of this would be vented. It was assumed particles exhausted would disperse such that there would be 1,700 potential infectious doses per liter at distances less than 2 meters from the stack, and 170 potential infectious doses per liter at distances 7 meters from the stack. Because

there are no dwellings within 500 meters of the stack and ultraviolet radiation from the sun would destroy infectious particles, it is concluded that this MCE would not pose a significant risk to the community. The *IDU Operations Manual* includes instructions for responding to emergency situations that may occur in the IDU, including the BL-3 laboratory.

The exposure of the technician breaking the flask was estimated at 5.4×10^5 infectious doses and coworkers coming immediately to the scene would be likewise exposed unless respiratory protection was used. Exposed workers would undergo prophylactic antibiotic treatment that would prevent disease and would undergo blood tests over a period of time to ensure that they were not infected. The *IDU Operations Manual* describes the emergency procedures required for decontamination in the event of an MCE.

5.2.11 Environmental Justice

Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority and Low Income Populations*, requires federal agencies to consider whether their projects will result in disproportionate adverse impacts on minority or low-income populations. The U.S. Census defines the poverty level as the income level, based on family size, age of householder, and the number of children under 18 years of age that is considered too low to meet essential living requirements without regard to the local cost of living. The U.S. Census considers a poverty area as an area in which at least 20% of the population lives below the poverty level. Implementation of the proposed action (Alternative I) is highly unlikely to result in significant adverse impacts to the human environment, including human health. Implementing Alternative II (no action) would eliminate the potential for adverse impacts.

5.3 CUMULATIVE IMPACTS

The CEQ regulations implementing NEPA define cumulative impacts to the environment as those effects resulting from the impact of the proposed action when combined with past, present, and future actions (40 CFR 1508.7). Thus, cumulative impacts are the sum of all direct and indirect impacts, both adverse and positive, that result from the incremental impacts of the action when added to other past, present, and predictable future actions regardless of source. Cumulative impacts may be accrued over time and/or impacts in conjunction with other pre-existing effects from other activities in the area (40 CFR 1508.25).

No negative cumulative environmental impacts have been observed from the conduct of activities similar to the proposed action at VPI&SU. It is highly unlikely that cumulative adverse environmental impacts will result from conducting the proposed research study (Alternative I). Contributions of the proposed study to the VPI&SU waste stream or resource utilization are negligible. The proposed research will be conducted in existing facilities and no construction or renovations are planned. Implementing Alternative II (no action) will eliminate the negligible adverse cumulative impacts associated with implementing the proposed action.

5.4 COMPARISON OF THE PROPOSED ACTION WITH THE ALTERNATIVE

5.4.1 Alternative I – Conduct Proposed Research at VPI&SU

The research methods, hazardous materials, safety, and containment practices employed in the conduct of the biological defense research for USAMRMC at VPI&SU are consistent with those required and employed at other biomedical research institutions performing similar work (DA, 1989; CDC/NIH, 1993). The potential for adverse impacts to the human environment resulting from the conduct of the proposed research is extremely small. Positive impacts to U.S. civilian and military are likely.

5.4.2 Alternative II – No Action

Alternative II, no-action, involves not conducting the proposed research at VPI&SU for USAMRMC. Implementing this alternative would eliminate the potential negligible adverse impacts associated with the proposed action. This is not preferred, however, because it would also eliminate the potential positive impacts associated with progress toward developing a safe and effective vaccine against human brucellosis.

6.0 CONCLUSIONS

The principal conclusions of this EA are: (1) the conduct of the proposed research project – *Expression of Brucella Antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice* (Alternative I, the preferred alternative) – is not expected to result in significant adverse environmental impacts; (2) implementing the preferred alternative will likely result in important benefits to the U.S. by enhancing progress toward developing an acceptable vaccine against human brucellosis; and (3) ceasing the proposed research project (Alternative II, no action) will eliminate the negligible environmental impacts associated with conducting the research, but it will also eliminate potentially significant advances in brucellosis vaccine development.

Laboratory work involving *Brucella* and vaccinia virus has been conducted at VPI&SU without significant environmental impact. The most severe potential effects associated with the proposed *Brucella* research are predicted to be negligible, and to date, all observed effects associated with these research activities have been insignificant. Potential risks to human health and the environment will continue to be mitigated by applying required standards, practices, and controls pertaining to the safe use and disposal of hazardous biological and chemical materials, the protection and conservation of natural resources, and the safe and ethical conduct of studies requiring animal subjects.

7.0 REFERENCES

Boyle, S.M. 1998a. Environmental assessment information request form from S.M. Boyle (VPI&SU).

Boyle, S.M. 1998b. Telephone conversation between S.M. Boyle (VPI&SU) and K.J. Romoser-Breno (BSA Environmental Services, Inc.) on May 19, 1998.

Boyle, S.M. 1998c. Telephone conversation between S.M. Boyle (VPI&SU) and B.A. Schaberg (BSA Environmental Services, Inc.) on April 30, 1998.

Capital Design and Construction Department, Virginia Polytechnic Institute and State University. 1998. Master Building List for Virginia Tech obtained from www.vt.edu.

Centers for Disease Control/National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories*. 1993.

Department of the Army. 1989. *Biological Defense Research Program Final Programmatic Environmental Impact Statement* (BDRP FPEIS).

Harding, L. and D.F. Liberman. 1995. Epidemiology of laboratory-associated infections. In: Laboratory Safety Principles and Practices, Second edition. Fleming, D.O., J.H. Richardson, I.J. Tulis, and D. Vesley (editors). ASM Press, Inc. Washington, DC.

Hawley, R. 1997. Trip report for the annual site inspection of Division of Biomedical Sciences and Pathobiology VPI&SU-SU.

Kaufmann, A.F. 1995. Telephone conversation between A.F. Kaufmann (Centers for Disease Control – Emerging Diseases) and J.R. Beaver (BSA Environmental Services, Inc.) on November 28, 1995.

Kaufmann, A.F. and J.M. Boyce. 1995. Transmission of bacterial and rickettsial zoonoses in the laboratory. In: Laboratory Safety Principles and Practices, Second edition. Fleming, D.O., J.H. Richardson, I.J. Tulis, and D. Vesley (editors). ASM Press, Inc. Washington, DC.

National Climatic Data Center. 1990. Historical weather data for Blacksburg, VA.

National Institutes of Health (NIH). 1997. *Guidelines for Research Involving Recombinant DNA Molecules.* National Institutes of Health, Department of Health and Human Services. October 1997.

Reay, K. 1998. Letter from K. Reay (Virginia Fish and Wildlife Information System) to K.J. Romoser-Breno (BSA Environmental Services, Inc.) dated April 15, 1998.

Roschelli, D. 1998. Telephone conversation between D. Roschelli (Virginia Tech Mechanical Services) and K.J. Romoser-Breno (BSA Environmental Services, Inc.) on April 14, 1998.

Schurig, G. 1998. Written communication between R. Carton (USAMRMC) and G. Schurig (VPI&SU) dated July 20, 1998.

Sewell, D.L. 1995. Laboratory-associated infections and biosafety. Clinical Microbiology Reviews 8:389-405.

Smiley, D. 1998. Telephone conversation between D. Smiley (VPI&SU Environmental Health and Safety Services) and B.A. Schaberg (BSA Environmental Services, Inc.) on May 19, 1998.

Soil Conservation Service. 1985. Soil Survey of Montgomery County, Virginia. U.S. Department of Agriculture and Virginia Polytechnic Institute and State University. September 1985.

U.S. Army Medical Research Institute of Chemical Defense. 1992. *The U.S. Army Medical Research Institute of Chemical Defense Environmental Assessment.* Final.

U.S. Environmental Protection Agency. 1998a. Upper Roanoke watershed data obtained from www.epa.gov/surf/hucinfo/03010101/.

U.S. Environmental Protection Agency. 1998b. Safe Drinking Water Information obtained from www.epa.gov/.

U.S. Fish and Wildlife Service. 1990. National Wetlands Inventory Map for Blacksburg, VA.

U.S. Geological Survey. 1983. Blacksburg Quadrangle Map.

University of Virginia Library of Social Sciences Data Center. 1998. County and City Data Books obtained from www.lib.virginia.edu/socsci/ccdb/.

Walter Reed Army Institute of Research. 1993a. *Walter Reed Army Institute of Research Environmental Assessment. Final.*

Walter Reed Army Institute of Research. 1993b. *Walter Reed Army Institute of Research, Leased Facilities, Environmental Assessment. Final.*

Walter Reed Army Institute of Research. 1994. *BSL-2 Vaccine Facility at the Walter Reed Army Institute of Research at Forest Glen, Maryland. Final.*

Virginia Department of Environmental Quality. 1998a. Regulatory Programs. Air Quality Data.

Virginia Department of Environmental Quality. 1998b. Blacksburg Area Air Quality Data.

Virginia Polytechnic Institute and State University, University Relations/Publications. 1998. University Profile obtained from www.vt.edu.

Virginia Tech Electric Services. 1998. Meter Readings Inquiry.

Virginia Polytechnic Institute and State University. 1996. Research Proposal. *Expression of Brucella antigens in Vaccinia Virus to Prevent Brucellosis in Humans: Protection Studies in Mice.* USAMRMC Log Number 96172001.

Young, D.E. 1996. Appendix F. Facility Safety Plan (FSP). VPI&SU Environmental Health and Safety Services. October 21, 1996.

8.0 PERSONS AND AGENCIES CONTACTED

Stephen M. Boyle, PhD	*Principal Investigator, Professor of Microbiology, VPI&SU*	(540) 231-4641
Marsha Caldwell	*Virginia Tech Electric Services*	(540) 231-6437
Robert J. Carton, PhD	*Environmental Coordinator, USAMRMC*	(301) 619-2004
David Dutton	*Director, Division of Project Review, Virginia Department of Historic Resources*	(804) 367-2323 x106
Bernadette Mondy	*Associate Director, Environmental, Health, and Safety Services, VPI&SU*	(540) 231-8758
Karen Reay	*FWIS Research Specialist Sr., Virginia Department of Game & Inland Fisheries, Fish and Wildlife Information Services*	(804) 367-1000
Don Roschelli	*Virginia Tech Mechanical Services*	(540) 231-9747
Doug Smiley	*Environmental Health and Safety Services, VPI&SU*	(540) 231-8758
Bruce Stoneman	*Soil Scientist, Natural Resources Conservation Service, USDA*	(804) 287-1640
Rebecca Wajda	*FWIS Coordinator, Virginia Department of Game & Inland Fisheries, Fish and Wildlife Information Services*	(804) 367-1000
Pat Worrell	*Office Manager, Virginia Tech Electric Services*	(540) 231-6437

9.0 LIST OF PREPARERS

The following personnel, under a USAMRMC contract to SAIC, provided instrumental technical assistance to USAMRMC in the preparation and review of this EA.

Technical Consultants:

Beth A. Schaberg MS, Biology	BSA Environmental Services, Inc. Beachwood, OH
John R. Beaver PhD, Environmental Engineering Sciences	BSA Environmental Services, Inc. Beachwood, OH
Kristin J. Romoser-Breno M.D.	BSA Environmental Services, Inc. Beachwood, OH

10.0 ACRONYMS AND ABBREVIATIONS

AR	Army Regulation
BDRP	Biological Defense Research Program
BL	biosafety level
BOC	Biotechnology Oversight Committee
CDC	Centers for Disease Control and Prevention
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations
cfu	colony forming unit
CHP	Chemical Hygiene Plan
CMMID	Center for Molecular Medicine and Infectious Diseases
DA	Department of the Army
DNA	deoxyribonucleic acid
DoD	Department of Defense
EA	Environmental Assessment
EHSS	Environmental, Health, and Safety Services
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FPEIS	Final Programmatic Environmental Impact Statement
GEM	genetically engineered microorganisms
HEPA	high-efficiency particulate air
IBC	Institutional Biosafety Committee
IDU	Infectious Disease Unit
MCE	Maximum Credible Event
Ml	milliliter
MSDS	Material Safety Data Sheet
MVA	modified vaccinia virus Ankara
NAAQS	National Ambient Air Quality Standards
NEPA	National Environmental Policy Act
NIH	National Institutes of Health
NPDES	National Pollutant Discharge Elimination System
NRC	Nuclear Regulatory Commission
OSHA	Occupational Safety and Health Administration
PCR	polymerase chain reaction
RCRA	Resource Conservation and Recovery Act
RNA	Ribonucleic acid
ROD	Record of Decision
RSC	Radiation Safety Committee
USAMRICD	U.S. Army Medical Research Institute of Chemical Defense
USAMRMC	U.S. Army Medical Research and Materiel Command
USC	U.S. Code
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
VDEQ	Virginia Department of Environmental Quality
VPI&SU	Virginia Polytechnic Institute and State University
WRAIR	Walter Reed Army Institute of Research
WR	Western Reserve

APPENDIX A: Detailed Description of Proposed Study Activities

Currently, there is no acceptable vaccine for preventing brucellosis in humans and the accepted method of preventing the disease in exposed individuals is treatment with antibiotic(s). If an individual were exposed to a genetically altered antibiotic-resistant form of *Brucella*, effective antibiotics might not be available to prevent disease. The purpose of the proposed research is to develop a vaccine to prevent brucellosis in soldiers exposed to antibiotic-resistant *Brucella* species. The proposed research involves using genetic engineering techniques to produce recombinant DNA with the ability to generate molecules (antigens) desirable for use in a *Brucella* vaccine. Antigens (e.g., proteins) stimulate the immune responses that are needed for developing immunity. The recombinant DNA molecules will contain DNA from vaccinia virus strains and from *Brucella* species. Two types of immunity are considered in the proposed research because *Brucella* affects several cell types (T lymphocytes, B lymphocytes, and macrophages) that function in different ways within the body. Humoral immunity refers to the production of antibodies (proteins specific to antigens) by the B lymphocyte immune cells. Cell-mediated immunity involves cells that do not produce antibodies, such as T lymphocytes.

Brucella is an intracellular pathogen and in infected animals is found in macrophages. These cells ingest and digest foreign particles and are involved in antigen presentation. Those macrophages activated by interferon gamma might have the capacity to destroy *Brucella*. The researchers seek to develop a vaccine containing *Brucella* genes that code for proteins that induce a strong cell-mediated immune response. The *Brucella* antigens required to achieve the necessary protective cell-mediated immunity have not been defined. Based on previous research, some *Brucella* antigens (e.g., *Brucella* HtrA protein) are known to induce a Th1 type of cell immune response that produces interferon gamma that enhances the protective response. *Brucella* antigens will be studied individually and in combination for their effectiveness in inducing an immune response.

A biological vector such as a virus is used to express genes in cells. Vaccinia virus was selected as the vector for *Brucella* genes because vaccinia virus replicates intracellularly, a vaccinia virus strain has been safely used in humans in the small pox vaccine, and a variety of *Brucella* proteins can be produced by vaccinia virus recombinants. *Brucella* genes in a vaccinia virus recombinant will be expressed (produce their antigens) intracellularly, similar to live *Brucella* replication in vivo.

Over the 2-year period of the research, the ability of various recombinants to produce immunity in mice will be studied. To compare immune responses, vaccinia virus/*Brucella* recombinants expressing one or more *Brucella* antigens will be constructed. In the mouse model, Western Reserve (WR) vaccinia virus strain replicates well and acts as a good expression vector. Some recombinants will be constructed using WR vaccinia virus expressing interleukin 12, because that cytokine plays a role in resistance to *Brucella* infection. The modified vaccinia virus Ankara (MVA) strain is unable to complete its replication cycle in humans while still expressing recombinant genes. This permits vaccination of immunodeficient humans. The MVA strain has been a safe and efficient means of inducing immune responses in mice. The MVA strain will be used later in the proposed research as an expression vector to develop recombinants with *Brucella* antigens found to induce protective responses in mice vaccinated with the WR strain recombinants.

Various plasmid transfer vectors obtained from the NIH will be used in the research to compare their effectiveness and toxicity. Three plasmid transfer vectors will be used with WR strains, including one vector with a synthetic early and late promoter. A fourth plasmid transfer vector will be used exclusively with the MVA strain.

The BALB/c mouse model will be used to test each recombinant for its ability to induce protective immunity. It is anticipated that 20 BALB/c mice will be required to test the immune response to each recombinant. An estimated total of 960 mice (including controls) will be used. Groups of five mice will

be vaccinated with low and high doses of each vaccinia virus/*Brucella* recombinant vaccine and with controls. Controls include vaccinia virus alone, vaccinia virus plasmid alone, *Brucella* vaccine RB51 (positive protection control), and saline (negative protection control). Some mice may be revaccinated. The mice will be challenged with virulent *Brucella abortus* or *Brucella melitensis* strains at 6 to 7 weeks after vaccination. At specified intervals, mouse sera will be tested for humoral antibody immune response by the Western blot method. The mice will be sacrificed 2 weeks after the challenge, at which time additional blood samples will be analyzed.

The humoral and cell-mediated immune responses to *Brucella* antigens will be analyzed. To assess immune protection, the mouse spleens will be cultured to determine the number of organisms as colony forming units (cfu). Clearance of *Brucella* from the spleen relative to the control is used to gauge effectiveness of the vaccine. To assess cell-mediated immunity, lymphocytes obtained from mouse spleens will be analyzed for lymphocyte proliferation and specific cytotoxic T cell activity using radioactive chromium (^{51}Cr) labeled *Brucella* infected syngeneic macrophage cell lines as targets. The enzyme-linked immunosorbent assay (ELISA) method will be used to test lymphocytes for production of the cytokines interferon gamma, interleukin 2, and interleukin 4. The corresponding cytokine messenger ribonucleic acid (RNA) induction will be analyzed using RT-PCR (polymerase chain reaction). *Brucella* antigens for use in the assays will be obtained using a pMAL expression/purification system to overexpress the *Brucella* genes in *Escherichia coli*.

The conduct of the proposed study will require eight personnel; two full-time (one postdoctoral fellow; one technician) and six part-time workers (four faculty members and two graduate students) (Boyle, 1998a). It is anticipated that seven people will be working with vaccinia virus (two full time and five part time) and eight with *Brucella* (two full-time and six part-time). There are 14 certified users of the biosafety level 3 (BL-3) suite (Boyle, 1998a).